UNIVERSITY OF PENNSYLVANIA

UNUSUAL ASPECTS OF MEIOTIC AND POSTMEIOTIC CHROMOSOMES OF GASTERIA

HSU-CHUAN TUAN

A THESIS

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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UNUSUAL ASPECTS OF MEIOTIC AND POSTMEIOTIC CHROMOSOMES OF GASTERIA[1]

HSU-CHUAN TUAN[2]

(WITH PLATES I, II AND FOURTEEN FIGURES)

Introduction

The investigation here described was initiated at the University of Pennsylvania in 1929. The preliminary work consisted of a study of the comparative action of different fixing reagents and of the methods of applying different nuclear stains. The investigation covered a wide range of plant material.

The technique of making cytological smears (20) rendered the question of fixation comparatively simple. Emphasis was therefore placed upon the acquiring of a satisfactory staining technique. It was found that picric acid destains material which has previously been treated with haematoxylin and safranin, and by its use the writer was able to get consistently satisfactory results. *Gasteria* was first investigated, and subsequently smears of *Clarkia*, *Solanum*, *Pinus*, and many others were studied experimentally. In the course of this study, a group of *Gasteria* smears, showing microsporogenesis, yielded an unusual phase of diakinesis chromosomes. This discovery led to the present report. In diakinesis, some cells showed a sudden change in their capacity for absorbing the stains, and in addition they were found to go through a regressive process to a false resting stage. Often the pollen mother cells of this particular plant divide three successive times, forming eight pollen grains.

TAYLOR (21, 23, 26) had previously made a thorough study of *Gasteria* chromosomes, and contributed much to our knowledge of their shape and structure. A comparable investigation of this series of abnormal chromosomes in the same genus might therefore be expected to reveal other interesting facts.

[1] Contribution from the botanical laboratory of the University of Pennsylvania.

[2] Fellow of the Chinese Educational Mission at Washington, D.C., of Tsing Hua University, Peiping, China.

A part of this work was done at the Marine Biological Laboratory, Woods Hole, in the summer of 1930, after the material had been collected and partially studied at the University of Pennsylvania. The research was then completed at the botanical laboratory of the University of Michigan. To these institutions the writer is much indebted for laboratory space and the necessary equipment.

Material and method

Pollen mother cells of *Gasteria* were collected in a greenhouse at the University of Pennsylvania in the spring of 1930. Young buds were teased out with a needle and the anthers transferred immediately to a thoroughly cleansed slide. With a stroke of a scalpel, the pollen mother cells were smeared evenly into a thin film on the slide, which was then submerged in the fixing fluid for 20 minutes. Many fixing fluids were tried, and although the results varied much in detail, the fixation of smears was generally better than preparations sectioned by the paraffin method. The following account is based upon the result obtained by the use of a special chromic-acetic-osmic fixative developed by TAYLOR (MCCLUNG 11) for *Gasteria* pollen mother cells. No paraffin preparations were used, since the smears give the most natural image of the cell, both in regard to its general contour and to the details of its chromosomes. Shrinkage is almost eliminated in most of the cells. The staining technique described by TUAN (**27, 28**) was followed, with some slight modification to suit each individual case. When haematoxylin was used, the picric acid differentiation was occasionally abbreviated by the slides being placed in a 0.5 per cent iron alum solution for 2 minutes before they were transferred to the picric acid for further differentiation. This saves the long schedule of differentiation required when picric acid alone is used. For *Gasteria*, however, the writer still prefers the long schedule of picric acid, but in the case of *Tradescantia*, prepared for purposes of comparison, a previous treatment in a 0.5 per cent iron alum solution cut down the time from 3 hours to 30 minutes. When stained with safranin, the slide was brought into a mixture of two parts of clove oil and one part of 95 per cent alcohol, and there the differentiation was further adjusted. Deeply stained or over stained slides can be saved by this process.[3] One example

[3] This alcohol-clove oil differentiation can also be applied to crystal violet smears

from each of the methods illustrates their general application to the *Gasteria* study:

	Minutes
Slide PK 8, Safranin	
1. Stained in Grubler's safranin O	5
2. Picric acid-alcohols	5
3. Ammonia-95% alcohol	0.5
4. Pure 95% alcohol	0.5
5. 95% alcohol-clove oil mixture	5–10
Slide KP 9, Haematoxylin[4]	
1. 2% iron alum	20
2. ½% haematoxylin	5
3. Differentiated in picric acid	160
Slide KP 6, Haematoxylin	
1. 2% iron alum	20
2. ½% haematoxylin	5
3. ½% iron alum	2
4. Differentiated in picric acid	30

Investigation

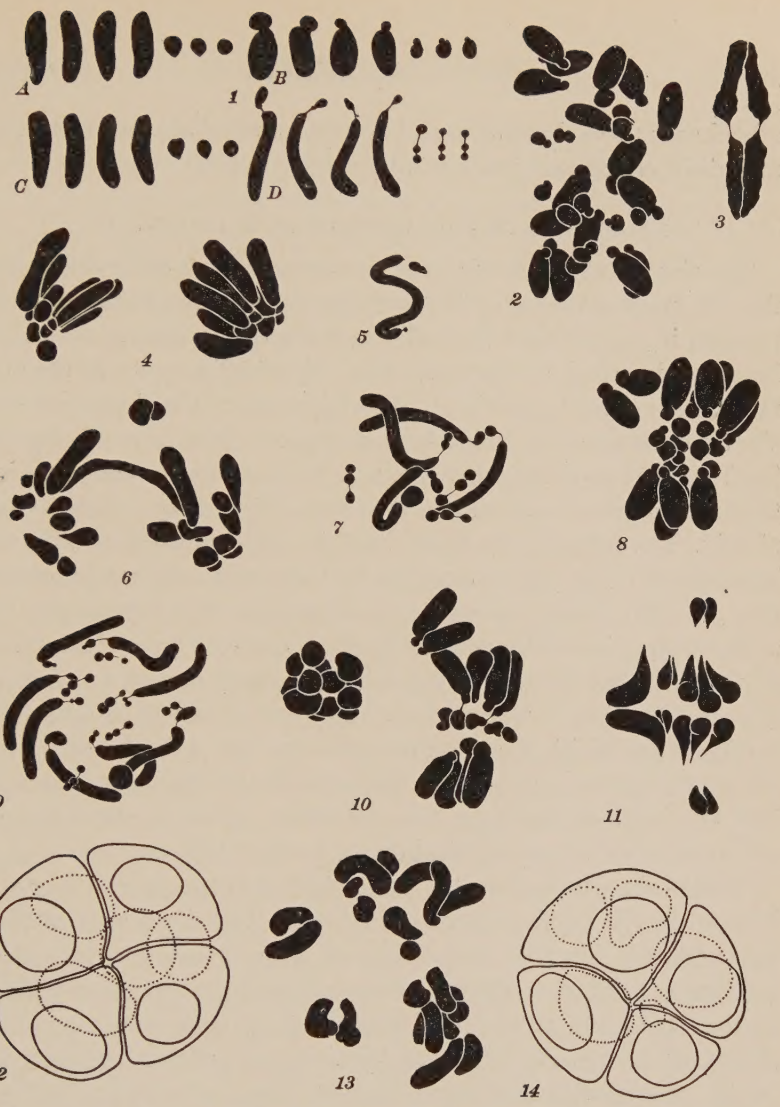
In the normal behavior of *Gasteria* chromosomes, according to Taylor (**21, 23, 26**), the first suggestion of prophase activity in meiosis is marked by an aggregation of chromatic granules, forming more or less spiral chromonemata. When the spirals straighten out from a tangled condition to form the extended chromatic threads, synapsis of homologous chromosome pairs occurs simultaneously at different places throughout the nucleus. The longitudinal split of the paired chromosomes, which form the tetrad, is accomplished before the diakinesis stage. Three pairs of pyriform chromosomes and four pairs of larger chromosomes are present on the metaphase plate. The tetrad condition of each pair is revealed distinctly. A coiled spiral of from seven to nine turns is present in the longer chromosomes, while in the small ones the number of coils is correspondingly reduced. These coils apparently are a result of the contraction of the more or less straight chromonemata of an earlier stage. In the anaphase, the two chromatids of each dyad may separate and the coils within them become more distinct. When telophase is reached, the chromosomal sheath is no longer stainable but

[4] When haematoxylin is used, each stage is followed by a prolonged washing in tap water.

is still present. Eventually the chromosomes become disorganized in the interphase. When they reappear for the second division, their shape and internal structure acquire interest from a new angle. At the proximal end of each chromosome a mild constriction is invariably present; perhaps in the three short ones a secondary constriction occurs along the shaft. In size, the homeotypic chromosomes are more slender and longer than the heterotypic ones. The internal structure of each of the homeotypic chromosomes resembles that of the somatic chromosomes, that is, each chromosome contains two chromonemata derived from a split of the coils. A quartet is formed as a result of two cell divisions. Each of the resulting cells divides again after the formation of a thick wall, typical of pollen grains.

There are several striking differences between the normal behavior of *Gasteria* chromosomes, as just described, and the peculiar mode of division to be recorded in the next few paragraphs.

The first sign of abnormality is a dispersal of the diakinesis chromosomes to form a false resting stage. More complicated abnormalities occur after the first metaphase. The nuclear membrane fails to form at telophase. Two sets of second metaphase chromosomes persist through the omission of the interphase and second prophase. The chromosomes thus produced assume two different aspects. The type (fig. 30) with distinct spiral structure is identical with that which appeared in the first metaphase; the other type (figs. 2, 8, 10, 18) is erratic and cannot be connected with any particular division of the chromosomes. So far as their outer form is concerned, they are intermediate between the homeotypic and heterotypic chromosomes, as they have extraordinary terminal heads and short, heavy bodies. Abnormal chromosomes of another type (fig. 9) are produced by some cells in which a nuclear membrane is formed after the telophase. The probable fate of these last was not determined, owing to a lack of sufficient material of this type, but the history of the chromosomes with spiral structure is traced to the formation of third division chromosomes within the nucleus of quartet cells (fig. 7). No regular division of the quartet was observed, but furrows of various depths occur in these cells, and have been noted in many preparations. This suggests that the third division is carried through by a simple process of furrowing. On the



FIGS. 1–14.—Fig. 1, selected chromosome sets showing four types of chromosomes: *A*, first metaphase chromosomes; *B*, second metaphase chromosomes with extraordinary heads; *C*, second metaphase chromosomes with shape and appearance of first metaphase chromosomes retained; *D*, set of constricted chromosomes. Fig. 2, second metaphase figure showing giant headed chromosomes scattered irregularly in cytoplasm; 28 elements counted in all, but only 26 shown. Fig. 3, pair of anaphase chromosomes showing bulging matrix. Fig. 4, early telophase showing partial rotation of daughter groups. Fig. 5, one of constricted chromosomes from binucleate cell showing satellite at lower end. Fig. 6, mid-anaphase figure showing partial rotation of daughter groups and non-separation of single chromatid. Fig. 7, third division chromosomes with all types of constrictions represented. Fig. 8, group of second metaphase chromosomes showing giant headed chromosomes and extruded dyad. Fig. 9, constricted chromosomes from binucleate cell. Fig. 10, two sets of giant headed chromosomes showing extruded dyad at periphery. Fig. 11, extrusion of small chromosome at equatorial plate stage. Fig. 12, 8-celled polyspore. Fig. 13, second metaphase figure showing extrusion of dyad from each of two sets of chromosomes. Fig. 14, 9-celled polyspore.

other hand, these chromosomes, like some of the diakinesis elements, may disintegrate and produce false resting nuclei.

Regression of chromosomes from diakinesis

The clearest evidence of tetrad chromosomes was observed during the diakinesis stage (fig. 16). From the general appearance of these threads, it seemed that the cell was perfectly normal, and had behaved as described by Taylor. The only peculiarity up to the first metaphase was a suggestion of disintegration of the thickened diakinetic chromosomes, which were recognized in a few cells by the fact that they stained faintly (fig. 17). When these chromosomes are traced through several stages, it is found that they collapse completely. The tetrad is gradually brought clearly into view with the homologous chromatids connected by numerous achromatic bridges (fig. 32). The chromosomes may be compared to a rope ladder. In a later stage the achromatic bridges are replaced by a clear space, so that parallel threads of an early prophase nature are formed (fig. 29). Further disintegration of the threads brings about a false resting stage, which might be mistaken for the earliest evidence of the first prophase, except that the nucleolus in this case is absent. Probably cells of this type will not divide again, as there was no evidence to show a reorganization for further division.

Those cells which show the same kind of disintegration but at a later stage will not be described in detail. A brief account of their development, however, may bring out some points for discussion. (1) Empty cells are frequently observed among all stages of meiotic figures. (2) Cells at the binucleate stage or quartet stage which show only a large but faintly stained nucleus are also frequent. (3) Nuclei of a quartet may show different stages of activity (fig. 40), sometimes from one to three nuclei of a quartet being missing. Whether these cells represent a complete breakdown of the chromatin material or not could not be determined, but from this example of the diakinetic chromosomes it seems likely. As to the cause of these abnormalities, it is rather rash to connect them with any one agent, such as pathological influence or genetical unbalance. It is most probable that there were other forces which, in addition to these, contributed much to the peculiar behavior of these cells.

First metaphase.—The lapse of time from diakinesis to metaphase is exceedingly short. This observation is confirmed by a general lack of intermediate stages in many preparations, although in a few cases figures of such were not hard to identify. When diakinesis ends, the chromosomes shorten and thicken with surprising rapidity. The scattered chromosomes are suddenly gathered up in the central portion of the cell, while the nuclear membrane disappears entirely and the fiber attachments are formed on each of the chromosomes. It seems that the chromosomes which are now not in contact with the cytoplasm are able to take up the stains more readily than at any other stage; therefore the metaphase chromosomes arranged on the equator have the appearance of solid rods, and their internal structure is detected only in well differentiated preparations. On the metaphase plate, seven pairs of chromosomes, split into tetrads (fig. 27), can be observed so far as the general contour of the cell and the chromosomes are concerned. There are, however, several striking cases of abnormal distribution. In a few cells one of the small chromosomes is found at the polar region when the rest of the set is still on the equator (fig. 11). Also, one of the small chromosomes is often extruded entirely from the spindle region (fig. 6), so that when migration is completed, it is left at the peripheral region of the equator where it rounds up quickly to form a small nucleus.

First anaphase.—The anaphase chromosomes can clearly be counted with seven dyad chromosomes in each daughter set. At the beginning of migration, the chromatids are evidently subjected to a strong pull between the spindle fibers and the surfaces of contact with their corresponding homologues. When they are midway between the pole and the equator, they are stretched to a certain extent, the turns of the spiral bulging out at places and suggesting the presence of an elastic matrix (fig. 3). Slender threads may be seen between them as the daughter chromatids are drawn farther and farther apart. As a rule, the partners of each dyad are separated before they reach the pole (fig. 31). In one extreme case (fig. 6) one of the chromatids was pulled across the equator and failed to separate. The telophase (fig. 4) follows, with a general lack of the compactness of the chromosome such as is noticed in the normal condition. The small chromosomes reach the pole ahead of the large

ones, although migration started simultaneously in all. Many cells now pass quickly to a second division without any of the phenomena associated with the heterotypic telophase. Only the least abnormal types, those which retain some evidence of telophase dispersal, will next be considered. However, a brief partial telophase may result in the formation of chromosomes as seen in figs. 2, 8, 10, and this will be described in connection with the second metaphase chromosomes.

Residual first telophase and derived chromosomes

The ends of the chromosomes toward the polar region approach each other, but the distal ends, extending toward the equator, alter their position very little. This is soon followed by a gradual rotation of the two groups of chromosomes in opposite directions (figs. 4, 6). After a complete turn of 90° they lose their staining ability. There are a few examples of nuclear membrane formation, but the majority of the cells do not show such a tendency. The former type of nuclei lead to another abnormal division, while the latter (fig. 24) soon recover their staining properties, so that they are seen as full sized chromosomes on the second metaphase plate (fig. 30). The interphase and second prophase of the latter type of nuclear division are entirely skipped, and consequently the appearance and structure of the chromosomes are unchanged from the first division, a condition similar to that of some animals in which the interphase and prophase are skipped during maturation divisions of the egg nucleus after entrance of the sperm.

In those cells in which the nuclear membrane plays a part, the chromosomes show a progressive loss of staining ability. The membrane appears first at one side, usually the side near the polar region (fig. 21), whence it grows about the mass to complete its formation (fig. 19). At this stage, as the nucleus increases in size, the chromatids are so lengthened that a continuous but loosely coiled spiral is seen in each (figs. 19, 21). Further stretching of the spirals (fig. 20 *a*, *b*, *c*) gives rise to an isolation of a single loop at the proximal end of each chromosome. Later the nuclear membrane, perhaps as a result of the excess lengthening of the chromosomes, is ill-defined and suggests that it is in the process of disintegration. Further evidence derived from the formation of heavily constricted

chromosomes (fig. 9) would seem to indicate that the nuclear membrane is finally and entirely disorganized. The cause of such a phenomenon was not determined, but it is interesting to note that immediately after disappearance of the membrane, two sets of constricted chromosomes are found in the cell. The origin of this type of chromosomes is probably that as soon as isolation of the tips of the spirals is accomplished (figs. 20, 23), anastomoses appear (figs. 37, 38). At first they are difficult to recognize, but later they assume a distinct threadlike form (fig. 9). This process is accompanied by the vacuolation characteristic of telophase chromosomes. The nuclear membrane is indefinite, and its complete collapse initiates reorganization of the chromosomes. A count of the elements in each of the cells reveals fourteen as the number (fig. 23). Two of them bear extremely big heads, which are further divided by another contraction (fig. 9). Six of them show comparatively small heads, while each of the six small chromosomes is terminated by a round head and has also a median constriction. All of these heads can be mistaken for satellites, as they are connected to the shafts by very slender threads. In one case, one of the large chromosomes showed a small satellite at the distal end (fig. 5). Presence of true satellites in meiotic chromosomes has not yet been reported, and their purely vegetative nature was emphasized by TAYLOR (23). No inference can be drawn from this sole exception.

SECOND METAPHASE.—It has already been mentioned that the interphase and prophase are skipped in many of the cells, owing to failure of the nuclear membrane to appear. This account of the behavior of these cells will therefore begin with the metaphase of the second division.

The chromosomes which retain the form they had in first division telophase have now recovered their staining ability in a surprising manner (fig. 24), each of the two sets of metaphase chromosomes being arranged on a second equator for the following division (fig. 30). The plane of this division is perpendicular to that of the first, as is shown by the rotation of the daughter groups in the earlier stages. From these they can clearly be seen as two sets, not arranged in a definite pattern. No case could be found in which the chromosomes were not widely separated from one another; this is

perhaps one of the reasons that a lagging and an extrusion of chromosomes will later occur. Rejection of a dyad chromosome was frequent, while the elimination of a dyad or two (fig. 13) from each daughter set of chromosomes was common. Those eliminated may give rise to some supernumerary spores.

Another type of abnormality is found at the second metaphase plate. Although many chromosomes show an internal spiral structure, there are some cells whose chromosomes show a striking difference (figs. 2, 8, 10, 18). These latter are constricted, with extraordinary heads at the tip, while the body is of a peculiar type, short and heavy. By an analysis of all the figures available, the origin of these chromosomes was traced back to the first telophase, when the nuclear membrane was just about to form. Consequently it is most probable that at telophase the nuclear membrane of these cells has a very brief existence, so that the chromosomes are only partially disorganized for the homeotypic division. Further collapse of the spindle would undoubtedly give rise to these scattered chromosomes (fig. 2). In fig. 2 the total count of the chromosomes is 28, but only 26 can be shown in the drawing. The count of 28 is exactly four times the haploid number. From the size of the heads, it is further determined that the total complex represents four sets of chromosomes, and each set (fig. 1 *b*) has the haploid number of seven. For these chromosomes at least, it shows that the chromatids of each tetrad are completely separated at late anaphase. Identified by the heads, these chromosomes are traced to the resting quartet stage. Late anaphase cells are frequently seen with four sets of chromosomes spread widely in the cytoplasm (fig. 26). Disintegration of the chromosomes, typical of telophase activity, occurs. Formation of a nuclear membrane is postponed until disintegration of the chromosomes is almost completed (fig. 33 *a–e*).

SECOND ANAPHASE.—The second early anaphase is brief. Cells are often observed in late anaphase (fig. 25) and telophase. It has been stated that the metaphase chromosomes are loosely scattered along the equatorial region, therefore it is to be expected that various abnormalities of the chromosomes would appear in the second anaphase. Several cases of chromosome extrusion from the spindle regions have been mentioned in connection with the metaphase chro-

mosomes, but as the appearance and history are similar to what has been described, a repetition will not be attempted.

Second telophase and third division chromosomes

In many cells, when the chromosomes have reached their respective poles, they round up to form the telophase. Ultimately a regular resting quartet is formed, all the chromosomes having lost their individuality within the nuclear membrane. Probably the meiotic activity of the cells developing in this manner is thereby concluded. There are a number of quartets, however, which retain the spirals throughout the telophase in perfect shape until a new set of active chromosomes is formed (fig. 7). Although these chromosomes were not observed to go through an elaborate division, their formation in the quartet nucleus is sufficient evidence for such a possibility. The history of the spirals here seems to be of the same nature as that shown in the first telophase, although the number in this case is reduced one-half. Furthermore, the constricted chromosomes formed after second telophase (fig. 9) are identical with those found after the first telophase. The development of these chromosomes could be only partially traced. It may be said, however, that at the end of the second telophase, the chromosomes gradually lost their chromaticity as the coiled spiral reappeared (figs. 28, 39). Each of the spirals then elongates at the expense of its width, so that the coils become very narrow compared with those first involved in this process. A loop or two at the tip of each spiral is then gradually dragged apart from the main body as the chromosomes reach their maximum length. The chromosome number is seven: one bears a big head (fig. 1 *d*), three are terminated by smaller heads, while at the tip of the small chromosomes a round head is shown with a secondary constriction along the shaft. This combination is exactly like that found after the first telophase, except that each type is represented by a single element. There is not much detail to be seen in these chromosomes. Their structure was discerned, however, in other cells in which the chromosomes were undergoing a process of regression. They had a single spiral. These chromosomes resemble the homeotypic ones in their constrictions, but they are different in structure, as the homeotypic chromosomes under normal condi-

tions have two spirals in each. The question arises as to whether or not this type of abnormality should be connected with the normal homeotypic chromosomes. The writer will develop this point later.

Several interesting figures may be observed in the regression quartets. The quartet nuclei in many cases show different stages of activity in the same group (fig. 40 *a-c*). A typical resting nucleus is shown in *b; c* is shown with partially disintegrated chromosomes; while *a* is intermediate. From the equal sized and equally stained nucleoli in these cells, it is probable that these stages are passed through in a short period, and the probable disturbance occurs immediately after formation of the third division chromosomes. Reformation of the nuclear membrane later will mark the beginning of the regression process. The most probable steps of chromosome behavior in these cells are as shown in fig. 22 *d*, *c*, *b*, *a*, and figs. 36, 40. The earliest stage of disintegration is marked by a loss of chromaticity of the chromosome matrix, so that the comparatively heavily stained spiral within the chromosomes is brought out in better contrast (fig. 22 *c*). The chromosome at the lower right hand corner of fig. 36, and two other segments in the same cell, show the spiral clearly, while the farther ends of these chromosomes are in a more advanced stage of disintegration. Increasing number of anastomoses and central vacuolation (fig. 22 *a*, *b*) bring the chromosomes close to a resting stage (fig. 40 *b*). It seems that the chromaticity of each spiral varies at regular intervals, and usually certain segments are still heavily stainable while others register little affinity for the stain (fig. 40 *a*), so nodules are distinctly shown at places throughout the nucleus. When vacuolization reaches a climax, the chromosomes appear double, with faintly stained peripheral regions and clear central cylinder (fig. 40 *b*). Besides these regression figures, there are two selected from a number of quartet cells worth mentioning. The one (fig. 34) with short spirals is rather difficult to assign to a particular developmental position. It is probably a second telophase figure, however, with the spirals segmented into short pieces. The other (fig. 35), with slender but continuous spirals, has been given no definite position either. There are four longer spirals and three shorter ones. If it is not one of the second telophase figures, it must be classified with the regression chromosomes, and should be followed closely by the spirals shown in fig. 36.

Polyspory

In several preparations, 8-celled spore groups are found among other abnormal groups containing from five to many cells in place of the normal quartet. It has been suggested that the lagging of chromosomes at first metaphase and at many other stages may produce extra nuclei, therefore all the supernumerary cells in any of these groups may be considered to be the natural consequence of such abnormalities. A detailed consideration of the 8-celled groups strengthens the assumption that third division does occur in some of the cells.

Eight nuclei of equal size frequently occur in 8-celled groups (fig. 12), while one or two small extra nuclei (fig. 14) are also frequently present. It is easy to account for the small nuclei as the result of misplaced chromosomes, but as to how the eight equal sized nuclei developed, there is only one reasonable explanation; that is, that they are the product of a third division. The evidence in support of the presence of a simple third division may be summed up in a few words. As soon as the chromosomes are formed in each of the four nuclei of a quartet, the nuclear membrane disappears, although the nucleolus, formed at the end of the telophase, is still obvious (fig. 7). The chromosomes, usually seven in number, spread apart in the cell without sign of spindle formation. The next stage, thus far observed, involves cleavage of various depths along the cell wall and cytoplasm. Whether this is the mode of the third division or not is without further proof, and the method by which these chromosomes are distributed to the daughter nuclei is another unanswered question. Additional work on the pollen mother cells of this plant may bring to light more evidence.

Rôle of nucleolus

From the resting cells up to the diakinesis stage, the nucleolus remains in the nucleus with little change. Its diameter is estimated as 2.5–3.5μ (figs. 15, 16). It disappears almost simultaneously with the nuclear membrane as the chromosomes are arranged for the first division metaphase. It is observed again as a faintly stained body at the end of the first telophase, with a much reduced diameter (1–1.5 μ). It increases in size within the nucleus containing the first type of constricted chromosomes. At this stage it measures

about 2 μ in diameter (fig. 9). With the other type of division, where the interphase and prophase are omitted, the nucleolus does not appear until the second telophase is far under way. It shows the same diameter as before; that is, it first appears as a body about 1 μ across, and then increases in bulk when the third division chromosomes are formed (fig. 7). Its definite function in this process cannot be accounted for; however, the interpretation that the nucleolus has some connection with the chromosomal appendages is eliminated. In this case it occurs simultaneously with the fully developed chromosomes, therefore it is not converted into any of these appendages.

Chromosome structure

Since the structure of chromosomes has already been discussed, only a few outstanding points will be added here. On the metaphase plate the chromosomes are heavily stained. From haematoxylin preparations, the spiral structure is not very distinct, but the number of coils was easily ascertained by the nodule-like structures which bulged out at places along the elastic matrix. It is estimated that in the longer chromosomes the spiral has from seven to nine turns, while in the small ones there are only two or three present. In many metaphase cells indication of a longitudinal split was not observed. The single nature is retained throughout until the formation of chromosomes with satellite-like appendages. As previously mentioned, this type of chromosome, in its outward appearance, resembles the normal homeotypic chromosome, but from its internal structure the resemblance is entirely lacking. The cause for such difference is probably due to the fact that the prophase, in which the split is supposed to take place, was skipped, and therefore the split was perforce omitted. The significance of this process is not understood, but it confirms the interpretation of Taylor (**26**) that the double spiral in the homeotypic chromosomes is the consequence of a longitudinal split completed at the second prophase. The internal structure of those chromosomes with giant heads is rather obscure. In many well differentiated cells, it is seen as a comparatively lighter central core with a densely stained peripheral region (fig. 18), containing well spaced nodules in two rows, a structure which makes the chromosomes appear double. When the chro-

mosomes come to the second anaphase (fig. 26) the same kind of apparent doubleness is maintained, in addition to the vacuoles and anastomoses.

Discussion and historical review

Several diverse problems are involved in the abnormal behavior of *Gasteria* chromosomes: (1) the occurrence of three successive divisions in microsporogenesis; (2) the appearance of the heterotypic type of chromosomes in the second division; (3) the exaggerated constrictions of the chromosomes; (4) the production of polyspores after the close of meiotic activity; (5) the regression of chromosomes from different stages of chromosomal development to a false resting stage; and (6) the structure of chromosomes.

Literature recording three or more divisions at the time of meiosis is scattered. Both in spore formation in Ascomycetes and oogenesis in Fucaceae, as well as in embryo sacs, it is well known that mitoses may occur after meiosis before the full number of cells is completed. The occurrence of the division at very different periods in the morphological life cycle suggests that the divisions resulting in polyspory are not unforeshadowed and altogether new in higher plants. Recently Stow (18), by applying a higher temperature to growing bulbs of *Hyacinthus*, obtained multinucleate giant pollen grains. He found that cells dividing three times in succession produce structures resembling embryo sacs, while those dividing four or five times give the same kind of irregularity but of a more complicated form. He concluded that the male potency in these cells is reduced by the physical means employed, and thus the female tendency is given a temporary dominance. Although this conclusion is hardly applicable to this case of *Gasteria*, where signs of embryo sac formation are entirely lacking, it suggests the possibility that material for this study was injured by some similar agents in the greenhouse before fixation.

As to the appearance of a similar type of chromosomes in the first and second division during meiosis, no corresponding case can be found among plants. Among certain forms of animals such a condition is of common occurrence, therefore it is rather hasty to establish any co-relation here.

Constrictions and satellites have been reported frequently as a

permanent morphological character for individual chromosomes. TAYLOR (21, 22, 23, 24, 25) identified certain chromosomes in many forms by the constrictions and satellites. SVESCHNIKOVA (19) classified the genus upon the size of chromosome heads and number of satellites seen in many species of *Vicia*. SAKAMURA (15) and many others, by means of the use of chloral hydrate and by following the constrictions, have settled the disputed counts in several plants and corrected the old conception of chromosomal tetrads and transverse divisions. However, the origin of such morphological characters is only partially discussed in these articles. NAWASCHIN (12) probably contributed the first interpretation that nucleolar material is responsible for the satellites. TAYLOR (22) and DARLINGTON (3) claimed that the satellite is the natural result of an extreme type of subterminal constriction. In the present case, it is seen that the satellite-like appendages are the direct production of isolated loops of chromatic spirals. Their coexistence with a nucleolus in many of the cells makes it obvious that the nucleolar material of *Gasteria* plays no direct part in the formation of such appendages.

There is an enormous amount of publication on polyspory. Almost without exception each article deals with hybrids or polyploid species. Taking the rose family for example, ERLANSON (4), in a study of American wild roses, found that polyspory is a common phenomenon among spontaneous hybrids or descendants of hybrids; SHOEMAKER (17) arrived at the same conclusion with species of cultivated apples; LONGLEY (9, 10) discovered the stability of diploid species in *Crataegus* and *Rubus*, while "The polyploid species are characterized by striking irregularities in chromosome distribution and irregular pollen-formation, leading frequently to polycary and polyspory." Investigations on *Potentilla* (14) and many related genera indicated the same results. In *Gasteria*, TAYLOR (21) has indicated the hybrid origin of much of the material in cultivation, and questioned the authenticity of specific names as commonly used. There remains little doubt that the species studied is by no means pure, and perhaps some of the abnormalities can be explained on account of genetic weaknesses.

In connection with the regression of chromosomes, experiments

by CHAMBERS (2) are especially interesting. He demonstrated by microdissection that the reversion of jelly-like chromosomes to a more or less homogeneous sol could be brought about by mechanical injury. With fixed material, degeneration of quartet nuclei is described in several cases. In *Melilotus alba*, ROGERS (13) and CASTETTER (1) reported disintegration of three nuclei in quartets and the formation of giant pollen grains by the fourth nucleus. In *Carex* the same condition of disintegration of certain quartet nuclei and giant pollen formation is given by JUEL (5). From these observations it may be inferred that disintegration of nuclei is a normal phenomenon in certain species, and in some others it may be the result of mechanical injury.

KAUFMANN (6, 7), KUWADA (8), SHARP (16), and TAYLOR (26) have already offered lengthy reviews on the structure of large chromosomes. The discovery of spiral chromonemata and interpretation controverting the older investigators have also been recorded many times. It now seems that the structure of the larger chromosomes is understood to a degree. What remains to be investigated is the structure of smaller chromosomes. With the aid of new and more accurate technique, it is certain that many of the observations of larger chromosomes will be linked together into a coherent interpretation when the smaller chromosomes begin to attract the attention of the more skilled workers.

So far as behavior of the chromosomes is concerned, the peculiar cases of *Gasteria* recorded here are exceedingly interesting as well as perplexing. In their natural environment, such unusual behavior is perhaps being reported for the first time. However, judging from the abnormalities induced by chloral hydrate (15), excessive heat (18), and mechanical injuries (2), it is possible that some physiological factors are involved. On the other hand, it is probable that the material used is from a certain hybrid species of *Gasteria*, as shown by the polyspory and lagging of chromosomes at different stages of chromosomal development. It would be interesting from both physiological and cytological points of view, therefore, to conduct some parallel experiments with well controlled factors, whereupon it would not be difficult to learn some of the causes of the disturbance.

Summary

1. Behavior of normal reduction chromosomes of *Gasteria* as described by TAYLOR is cited and summarized.

2. Three types of abnormal meiotic and postmeiotic chromosomes of the same plant are described. One type, represented by closely coiled second metaphase chromosomes, is directly derived from the heterotypic chromosomes by an omission of the first interphase and second prophase. The other type, represented by giant headed chromosomes, is derived from the first metaphase chromosomes through a partial telophasic disorganization. The third type, represented by heavily constricted chromosomes in binucleated cells, is derived from the first metaphase chromosomes through a short lived interphase and breakdown of the nuclear membrane. The constrictions are much exaggerated and resemble the satellites in the somatic chromosomes.

3. The spiral which is involved in the structure of these types is single through the omission of the first interphase and second prophase.

4. Formation of third division chromosomes and 8-celled groups is described. This condition is thus far unique in higher plants.

5. Abnormal diakinetic chromosomes and chromosomes at other stages are able to assume a false resting stage after a brief process of disintegration of the chromatic material.

6. A nucleolus was seen in nuclei with fully developed chromosomes; it was not converted into any of the chromosomal appendages.

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EXPLANATION OF PLATES I, II

All drawings were made with the aid of Zeiss 1.5 mm. apochromatic objective and ×20 compensation ocular, giving an approximate magnification of 4500.

PLATE I

FIG. 15.—Open spireme nucleus.

FIG. 16.—Nucleus at diakinesis stage.

FIG. 17.—Segment of diakinetic chromosomes showing first sign of regression.

FIG. 18.—Giant headed chromosomes showing structural detail.

FIG. 19.—Mid-telophase chromosomes showing loosely connected spirals.

FIG. 20.—First telophase chromosomes (*a*, *b*, *c*) showing origin of constrictions.

FIG. 21.—Early telophase nucleus showing loosening of spirals and formation of nuclear membrane at side near polar region.

FIG. 22.—Third division chromosomes (*a*, *b*, *c*, *d*) showing process of regression.

FIG. 23.—Late telophase spiral showing anastomoses and vacuolation.

FIG. 24.—Two sets of spiral chromosomes just before second metaphase.

FIG. 25.—Second anaphase of spiral chromosomes.

FIG. 26.—Second anaphase of giant headed chromosomes.

PLATE II

FIG. 27.—First metaphase chromosomes at equatorial plate stage.

FIG. 28.—Spirals in quartet nucleus, seven can be counted.

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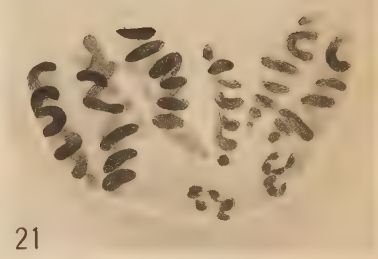
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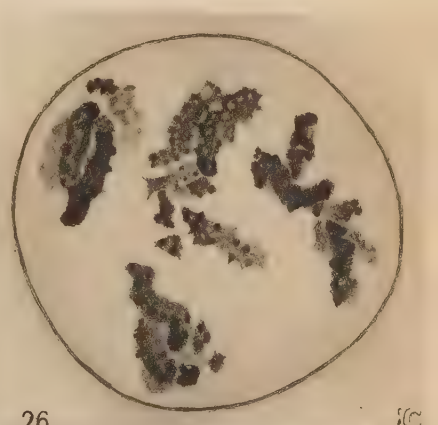
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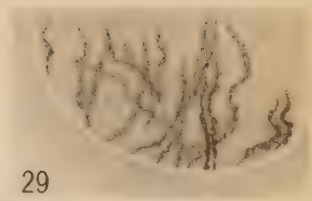
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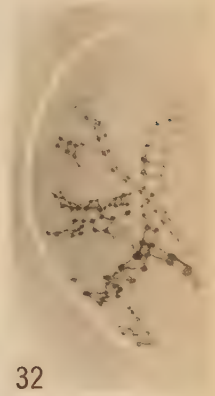
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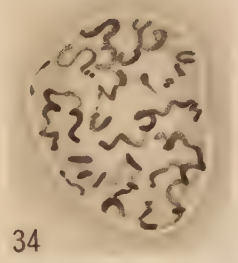
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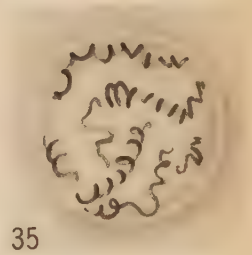
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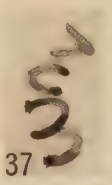
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FIG. 29.—Chromatic thread from regression diakinetic chromosome.

FIG. 30.—Two sets of second metaphase dyads derived directly from first metaphase tetrads showing spiral structure in each of chromatids.

FIG. 31.—Late anaphase daughter group showing separation of dyad partners.

FIG. 32.—Tetrad threads from regression diakinetic chromosomes.

FIG. 33.—Selected second telophase giant headed chromosomes (*a*, *b*, *c*, *d*, *e*) showing disorganization of chromatin material; head still recognizable in *a*.

FIG. 34.—Spirals from quartet nucleus.

FIG. 35.—Seven continuous spirals from another quartet nucleus.

FIG. 36.—Quartet nucleus showing regression of third division chromosomes.

FIG. 37.—First telophase spiral showing anastomoses.

FIG. 38.—Same as fig. 37.

FIG. 39.—Quartet nucleus at early telophase, seven spirals can be counted.

FIG. 40.—Same as fig. 36; *b* shown at resting stage.